

CLAIMS

- 1 A sensitive proteasome sensor which can detect proteasome activity levels in a cell or organism comprising a construct including;
 - a) an indicator molecule, and
 - b) a protein degradation tag.
- 2 The construct of claim 1, wherein said construct is a nucleic acid sequence.
- 3 The construct of claim 2, wherein said construct is a DNA sequence.
- 4 The construct of claim 3, wherein said construct is provided in a vector to facilitate transfer to a cell of interest.
- 5 The construct of claim 4, wherein said vector is a plasmid or viral vector.
- 6 The construct of claim 3, wherein said construct is a RNA sequence.
- 7 The construct of claim 1, wherein said construct is a protein.
- 8 The construct of claim 1, wherein the reporter is observable in an intact cell or organism of interest.
- 9 The construct of claim 8, wherein the reporter is a florescent protein.
- 10 The construct of claim 9, wherein the reporter is a reef coral fluorescent protein.
- 11 The construct of claim 10, wherein the report is selected from the group comprising (RCFPs) ZsGreen, ZsYellow, AmCyan, AsRed (red), DsRed (orange-red) and HcRed (far-red).
- 12 The construct of claim 9, wherein the reporter is from an medusa organism.
13. The construct of claim 9, wherein the reporter is a GFP, or a GFP mutant such as eGFP

14 The construct of claim 9, wherein the reporter is selected from a fluorescent protein a delay of 1-20 hours before the appearance of fluorescence in the cell

15 The construct of claim 14, wherein the reporter is selected from a fluorescent protein a delay of 1-6 hours before the appearance of fluorescence in the cell

16 The construct of claim 15, wherein the reporter is selected from a fluorescent protein a delay of 1-3 hours before the appearance of fluorescence in the cell

17 The construct of Claim 1, wherein the a protein degradation tag is specific to one of the following group, pest system, ubiquitin system, or other systems.

18 The construct of Claim 17, wherein the tag is specific to the pest system.

19 The construct of Claim18, wherein the tag is selected from E1A (residues 44 to 49, 125-149, 177-202, 223-244), c-myc (10-51, 52-65, 83-126, 168-206, 206-241, 241-269, 276-287), p53 (39-62, 62-98, 213-232), c-Fos (31-91, 128-139, 205-250, 265-279, 307-358, 360-380), v-Myb (4-16, 174-186), p730 (323-361), HSP70 (33-46, 125-152, 424-445), HMG-CoA reductase (381-395, 429-442, 442-456), TAT (382-395), alpha-casein (58-79, 151-193), beta-casein (1-25, 113-134)

20. The construct of Claim 18, wherein said tag is rich in one or more of the following, proline (P), glutamic acid (E), serine (S) and threonine (T)

21. The construct of Claim17, wherein a minimally effective tag is augmented by additional length of sequences upstream and/or downstream of the sequence. include about 1-100 aa additions to either or both side of the targeting sequence, with about 5-50 aa additions being the preferred range, and about 8-20 being the most preferred range.

22 The construct of Claim 1, wherein the a protein degradation tag is specific to the ubiquitin system.

23 The construct of Claim 22, wherein the tag is selected from

Cyclins A, B, D, E, CDK inhibitors, p53, c-fos, c-Jun, c-myc, N-myc, Ikb, p130, cdc25 phosphatase, TAT, Topoisomerase I and IIalpha.

24 The construct of Claim 1 wherein specific proteasome effecting proteasome activity is determined to a high sensitivity.

25 The construct of Claim 24, wherein detection of Epoxomicin levels are provided at 6 hours of exposure at about 1-30 nM, preferably about 1-10 nM, and most preferably about 1-5 nM

26 The construct of Claim 24, wherein detection of Lactacystin levels are provided at 6 hours at about 10-500 nM, preferably about 50-300 nM and most preferably about 100-200 nM.

27 The construct of Claim 24, wherein detection of ZLLH levels are provided at 6 hours at about 1-200 nM, preferably at about 5-50 nM, and most preferably at 10-20 nM.

28 The construct of Claim 24, wherein detection of ALLN levels are provided at 6 hours at about 0.05-2.5 uM, preferably 0.1-1.5 uM, and most preferably 0.2-1.0 uM.

29 The construct of claim 1, where said sensor is employed for one or more of the following, high throughput screening for proteasome inhibitors, screening for

proteasome targeting motifs, multiplexed assays, concomitant assay for proteasome and FP Effects, and analysis of endoproteolytic systems.